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1.	Your reference	40322/JMD
2.	Patent application number *(The Patent Office will fill in this part)*	**9827104.2** 10 DEC 1998
3.	Full name, address and postcode of the or of each applicant *(underline all surnames)*	ONYVAX LIMITED, P.O. Box 17717 St. Georges Hospital Medical School, Cranmer Terrace, London SW17 0WG.
	Patents ADP number *(if you know it)*	7566284001
	If the applicant is a corporate body, give the country/state of incorporation	United Kingdom
4.	Title of the invention	New Cancer Treatments
5.	Full name, address and postcode in the United Kingdom to which all correspondence relating to this form and translation should be sent	Reddie & Grose 16 Theobalds Road LONDON WC1X 8PL
	Patents ADP number *(if you know it)*	91001
6.	If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number	Country / Priority application *(If you know it)* / Date of filing *(day/month/year)*
7.	If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application	Number of earlier application / Date of filing *(day/month/year)*
8.	Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:* *a) any applicant named in part 3 is not an inventor, or* *b) there is an inventor who is not named as an applicant, or* *c) any named applicant is a corporate body.* *See note (d))*	YES

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Continuation sheets of this form	
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Claim*(s)*	2
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Priority documents	0
Translations of priority documents	0
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	0
Request for preliminary examination and search *(Patents Form 9/77)*	1
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11. I/We request the grant of a patent on the basis of this application.

Signature: Reddie & Grose

Date: 9 December 199

12. Name and daytime telephone number of person to contact in the United Kingdom

J M DAVIES
01223-360350

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NEW CANCER TREATMENTS

Field of the Invention

This invention is concerned with agents for the treatment of primary, metastatic and residual cancer in mammals (including humans) by inducing the immune system of the mammal or human afflicted with cancer to mount an attack against the tumour lesion. In particular, the invention pertains to the use of whole-cells, derivatives and portions thereof with or without vaccine adjuvants and/or other accessory factors. More particularly, this disclosure describes particular combinations of whole-cells and derivatives and portions thereof that form the basis of treatment strategy.

Background to the Invention

It is known in the field that cancerous cells contain numerous mutations, qualitative and quantitative, spatial and temporal, relative to their normal, non-cancerous counterparts and that at certain periods during tumour cells' growth and spread a proportion of these are capable of being recognised by the hosts' immune system as abnormal. This has led to numerous research efforts world-wide to develop immunotherapies that harness the power of the hosts' immune system and direct it to attack the cancerous cells, thereby eliminating such aberrant cells at least to a level that is not life-threatening (reviewed in Maraveyas, A. & Dalgleish, A.G. 1997 *Active immunotherapy for solid tumours in vaccine design* in The Role of Cytokine Networks, Ed. Gregoriadis *et al.*, Plenum Press, New York, pages 129-145; Morton, D.L. and Ravindranath, M.H. 1996 *Current concepts concerning melanoma vaccines* in Tumor Immunology - Immunotherapy and Cancer Vaccines, ed. Dalgleish, A.G. and Browning, M., Cambridge University Press, pages 241-268. See also other papers in these publications for further detail).

Numerous approaches have been taken in the quest for cancer immunotherapies, and these can be classified under five categories:

Non-specific immunotherapy

Efforts to stimulate the immune system non-specifically date back over a century to the pioneering work of William Coley (Coley, W.B., 1894 Treatment of inoperable malignant tumours with toxins

of erisipelas and the Bacillus prodigosus. Trans. Am. Surg. Assoc. 12: 183). Although successful in a limited number of cases (e.g. BCG for the treatment of urinary bladder cancer, IL-2 for the treatment of melanoma and renal cancer) it is widely acknowledged that non-specific immunomodulation is unlikely to prove sufficient to treat the majority of cancers. Whilst non-specific immune-stimulants may lead to a general enhanced state of immune responsiveness, they lack the targeting capability and also subtlety to deal with tumour lesions which have many mechanisms and plasticity to evade, resist and subvert immune-surveillance.

Antibodies and monoclonal antibodies

Passive immunotherapy in the form of antibodies, and particularly monoclonal antibodies, has been the subject of considerable research and development as anti-cancer agents. Originally hailed as the magic bullet because of their exquisite specificity, monoclonal antibodies have failed to live up to their expectation in the field of cancer immunotherapy for a number of reasons including immune responses to the antibodies themselves (thereby abrogating their activity) and inability of the antibody to access the lesion through the blood vessels. To date, three products have been registered as pharmaceuticals for human use, namely *Panorex* (Glaxo-Wellcome), *Rituxan* (IDEC/Genentech/Hoffman la Roche) and *Herceptin* (Genentech/Hoffman la Roche) with over 50 other projects in the research and development pipeline. Antibodies may also be employed in active immunotherapy utilising anti-idiotype antibodies which appear to mimic (in an immunological sense) cancer antigens. Although elegant in concept, the utility of antibody-based approaches may ultimately prove limited by the phenomenon of 'immunological escape' where a subset of cancer cells in a mammalian or human subject mutates and loses the antigen recognised by the particular antibody and thereby can lead to the outgrowth of a population of cancer cells that are no longer treatable with that antibody.

Subunit vaccines

Drawing on the experience in vaccines for infectious diseases and other fields, many researchers have sought to identify antigens that are exclusively or preferentially associated with cancer cells, namely tumour specific antigens (TSA) or tumour associated antigens (TAA), and to use such antigens or fractions thereof as the basis for specific active immunotherapy.

There are numerous ways to identify proteins or peptides derived therefrom which fall into the category of TAA or TSA. For example, it is possible to utilise differential display techniques

whereby RNA expression is compared between tumour tissue and adjacent normal tissue to identify RNAs which are exclusively or preferentially expressed in the lesion. Sequencing of the RNA has identified several TAA and TSA which are expressed in that specific tissue at that specific time, but therein lies the potential deficiency of the approach in that identification of the TAA or TSA represents only a "snapshot" of the lesion at any given time which may not provide an adequate reflection of the antigenic profile in the lesion over time. Similarly a combination of cytotoxic T lymphocyte (CTL) cloning and expression-cloning of cDNA from tumour tissue has lead to identification of many TAA and TSA, particularly in melanoma. The approach suffers from the same inherent weakness as differential display techniques in that identification of only one TAA or TSA may not provide an appropriate representation of a clinically relevant antigenic profile.

Over fifty such subunit vaccine approaches are in development for the treatment of a wide range of cancers, although none has yet received marketing authorisation for use as a human pharmaceutical product. In a similar manner to that described for antibody-based approaches above, subunit vaccines may also be limited by the phenomenon of immunological escape.

Gene therapy

The majority of gene therapy trials in human subjects have been in the area of cancer treatment, and of these a substantial proportion have been designed to trigger and/or amplify patients' immune responses. Of particular note in commercial development are Allovectin-7 and Leuvectin, being developed by Vical Inc for a range of human tumours, CN706 being developed by Calydon Inc for the treatment of prostate cancer, and StressGen Inc.'s stress protein gene therapy for melanoma and lung cancer. At the present time, it is too early to judge whether these and the many other 'immuno-gene therapies' in development by commercial and academic bodies will ultimately prove successful, but it is widely accepted that commercial utility of these approaches are likely to be more than a decade away.

Cell-based vaccines

Tumours have the remarkable ability to counteract the immune system in a variety of ways including: downregulation of the expression of potential target proteins; mutation of potential target proteins; downregulation of surface expression of receptors and other proteins; downregulation of MHC class I and II expression thereby disallowing direct presentation of TAA or TSA peptides; downregulation of co-stimulatory molecules leading to incomplete stimulation of T-cells leading to

anergy; shedding of selective, non representative membrane portions to act as decoy to the immune system; shedding of selective membrane portions to anergise the immune system; secretion of inhibitory molecules; induction of T-cell death; and many other ways. What is clear is that the immunological heterogeneity and plasticity of tumours in the body will have to be matched to a degree by immunotherapeutic strategies which similarly embody heterogeneity. The potential advantages are:

(a) whole cells contain a broad range of antigens, providing an antigenic profile of sufficient heterogeneity to match that of the lesions as described above;

(b) being multivalent (i.e. containing multiple antigens), the risk of immunological escape is reduced (the probability of cancer cells 'losing' all of these antigens is remote); and

(c) cell-based vaccines include TSAs and TAAs that have yet to be identified as such; it is possible if not likely that currently unidentified antigens may be clinically more relevant than the relatively small number of TSAs/TAAs that are known.

Cell-based vaccines fall into two categories. The first, based on autologous cells, involves the removal of a biopsy from a patient, cultivating tumour cells *in vitro*, modifying the cells through transfection and/or other means, irradiating the cells to render them replication-incompetent and then injecting the cells back into the same patient as a vaccine. Although this approach enjoyed considerable attention over the past decade, it has been increasingly apparent that this individually-tailored therapy is inherently impractical for several reasons. The approach is time consuming (often the lead time for producing clinical doses of vaccine exceeds the patients' life expectancy), expensive and, as a 'bespoke' product, it is not possible to specify a standardised product (only the procedure, not the product, can be standardised and hence optimised and quality controlled). Furthermore, the tumour biopsy used to prepare the autologous vaccine will have certain growth characteristics, interactions and communication with surrounding tissue that makes it somewhat unique. This alludes to a potentially significant disadvantage to the use of autologous cells for immunotherapy: a biopsy which provides the initial cells represents an immunological snapshot of the tumour, in that environment, at that point in time, and this may be inadequate as an immunological representation over time for the purpose of a vaccine with sustained activity that can be given over the entire course of the disease.

The second type of cell-based vaccine and the subject of the current invention describes the use of allogeneic cells which are genetically (and hence immunologically) mismatched to the patients. Allogeneic cells benefit from the same advantages of multivalency as autologous cells. In addition, as allogeneic cell vaccines can be based on immortalised cell lines which can be cultivated indefinitely *in vitro,* thus this approach does not suffer the lead-time and cost disadvantages of autologous approaches. Similarly the allogeneic approach offers the opportunity to use combinations of cells types which may match the disease profile of an individual in terms of stage of the disease, the location of the lesion and potential resistance to other therapies.

There are numerous published reports of the utility of cell-based cancer vaccines (see, for example, Dranoff, G. *et al.* WO 93/06867; Gansbacher, P. WO 94/18995; Jaffee, E.M. *et al.* WO 97/24132; Mitchell, M.S. WO 90/03183; Morton, D.M. *et al.* WO 91/06866). These studies encompass a range of variations from the base procedure of using cancer cells as an immunotherapy antigen, to transfecting the cells to produce GM-CSF, IL-2, interferons or other immunologically-active molecules and the use of 'suicide' genes. Groups have used allogeneic cell lines that are HLA-matched or partially-matched to the patients' haplotype and also allogeneic cell lines that are mismatched to the patients' haplotype in the field of melanoma and also mismatched allogeneic prostate cell lines transfected with GM-CSF.

Description of the Invention

The invention disclosed here relates to a product comprised of a cell line or lines intended for use as an allogeneic immunotherapy agent for the treatment of cancer in humans and other mammals. All of the studies of cell-based cancer vaccines to date have one feature in common, namely the intention to use cells that contain at least some TSAs and/or TAAs that are shared with the antigens present in patients' tumour. In each case, tumour cells are utilised as the starting point on the premise that only tumour cells will contain TSAs or TAAs of relevance, and the tissue origins of the cells are matched to the tumour site in patients.

A primary aspect of the invention is the use of immortalised normal, non-malignant cells as the basis of an allogeneic cell cancer vaccine. Normal cells do not posses TSAs or relevant concentrations of TAAs and hence it is surprising that normal cells as described herein are effective as anti-cancer vaccines. The approach is general and can be adapted to any mammalian tumour by the use of immortalised normal cells derived from the same particular tissue as the tumour intended to be treated. Immortalised normal cells can be prepared by those skilled in the art using published methodologies, or they can be sourced from cell banks such as ATCC or ECACC, or they are available from several research groups in the field.

For prostate cancer, for example, a vaccine may be based on one or a combination of different immortalised normal cell lines derived from the prostate which can be prepared using methods reviewed and cited in Rhim, J.S. and Kung, H-F., 1997 Critical Reviews in Oncogenesis 8(4):305-328 or selected from PNT1A (ECACC Ref No: 95012614), PNT2 (ECACC Ref No: 95012613) or PZ-HPV-7 (ATCC Number: CRL-2221).

A further aspect of the invention is the addition of TSAs and/or TAAs by combining one or more immortalised normal cell line(s) with one, two or three different cell lines derived from primary or metastatic cancer biopsies.

All the appropriate cell lines will show good growth in large scale cell culture and sufficient characterisation to allow for quality control and reproducible production.

The cell lines are lethally irradiated utilising gamma irradiation at 20-400 Gy to ensure that they are

replication incompetent prior to use in the mammal or human.

The cell lines and combinations referenced above, to be useful as immunotherapy agents must be frozen to allow transportation and storage, therefore a further aspect of the invention is any combination of cells referenced above formulated with a cryoprotectant solution. Suitable cryoprotectant solutions may include but are not limited to, 10-30% v/v aqueous glycerol solution, 5-20% v/v dimethyl sulphoxide or 5-20% w/v human serum albumin may be used either as single cryoprotectants or in combination.

A further embodiment of the invention is the use of the cell line combinations with non-specific immune stimulants such as BCG or M. Vaccae, Tetanus toxoid, Diphtheria toxoid, Bordetella Pertussis, interleukin 2, interleukin 12, interleukin 4, interleukin 7, Complete Freund's Adjuvant, Incomplete Freund's Adjuvant or other non-specific agents known in the art. The advantage is that the general immune stimulants create a generally enhanced immune status whilst the combinations of cell lines, both add to the immune enhancement through their haplotype mismatch and target the immune response to a plethora of TAA and TSA as a result of the heterogeneity of their specific origins.

Example 1

An immortalised cell line derived from normal prostate tissue namely PNT2 was grown in roller bottle culture in RPMI 1640 media supplemented with 2 mM L-glutamine and 5% foetal calf serum (FCS) following recovery from liquid nitrogen stocks. Following expansion in T175 static flasks the cells were seeded into roller bottles with a growth surface area of 850 cm^2 at 1-20 $x10^7$ cells per roller bottle

Two secondary derived cell lines were also used, namely PC3 and Du145 both of which were sourced from the ATCC collection. PC-3 was grown in large surface area static flasks in Coons modified Hams F12 media supplemented with 7% FCS and 2 mM L-glutamine following seeding at 1-$10x10^6$ cells per vessel and then grown to near confluence. Du-145 was expanded from frozen stocks in static flasks and then seeded into 850 cm^2 roller bottles at 1-$20x10^7$ cells per bottle and grown to confluence in DMEM medium containing 10% FCS and 2 mM L-glutamine.

All cell lines were harvested utilising trypsin at 1x normal concentration. Following extensive washing in DMEM the cells were re-suspended at a concentration of 5-40x10^6 cells/ml and irradiated at 20-400 Gy using a Co^{60} source. Following irradiation the cells were formulated in cryopreservation solution composing of 10% DMSO, 8% human serum albumin in phosphate buffered saline, and frozen at a cell concentration of 5-150 x10^6 cells/ml, in liquid nitrogen until required for use.

The cells were warmed gently in a water bath and either used directly or admixed with a suitable adjuvant prior to injection into patients. Injections were made intra-dermally at four injection sites into the draining lymph nodes.

Example 2

An immunotherapeutic agent for the treatment of prostate cancer containing lethally irradiated immortal prostate cell lines comprising:

normal cell lines PNT-1 and/or PNT-2 and/or PZ-HPV-7;

alone or together with any or all of one or more cell lines selected from: primary cell lines SHMAC1, SHMAC2, SHMAC5, SHMAC6, P4E6, NIH1519-CPTX, NIH1532-CP2TX, NIH1535-CP1TX or NIH1542-CP3TX;

secondary cell lines LnCap, DU145 and PC3;

alone or together with a non-specific immunostimulant such as BCG, M. vaccae, Tetanus toxoid, Diphtheria toxoid, interleukin 2, interleukin 12, interleukin 4, interleukin 7 or other agents known in the art.

Claims

1. An allogeneic immunotherapy agent for the treatment of cancer in humans and other mammals, the agent being an anti-cancer vaccine which consists of or comprises immortalised normal, non-malignant human or other mammalian tissue cells.

2. An agent according to claim 1 wherein the vaccine is adapted to any mammalian tumour by the use of immortalised normal cells derived from the same particular tissue as the tumour intended to be treated.

3. An agent according to claim 1 or 2 for prostate cancer treatment, wherein the vaccine is based on one or a combination of different immortalised normal cell lines derived from the prostate.

4. An agent according to claim 3 wherein the normal cell lines are selected from PNT1A (ECACC Ref No: 95012614), PNT2 (ECACC Ref No: 95012613) and PZ-HPV-7 (ATCC Number: CRL-2221).

5. An agent according to any preceding claim additionally including TSAs and/or TAAs by combining one or more immortalised normal cell line(s) with one, two or three different cell lines derived from primary or metastatic cancer biopsies.

6. An agent according to any preceding claim wherein the cell lines are lethally irradiated utilising gamma irradiation at 20-400 Gy to ensure that they are replication incompetent prior to use in the mammal or human.

7. An agent according to any preceding claim wherein the combination of cells is formulated with a cryoprotectant solution.

8. An agent according to any preceding claim wherein the cryoprotectant solution may include 10-30% v/v aqueous glycerol solution, 5-20% v/v dimethyl sulphoxide or 5-20% w/v human serum albumin may be used either as single cryoprotectants or in combination.

9. An agent according to any preceding claim additionally including one or more non-specific immune stimulants such as BCG or M. Vaccae, Tetanus toxoid, Diphtheria toxoid, Bordetella Pertussis, interleukin 2, interleukin 12, interleukin 4, interleukin 7, Complete Freund's Adjuvant, Incomplete Freund's Adjuvant.

10. An immunotherapeutic agent for the treatment of prostate cancer containing lethally irradiated immortal prostate cell lines comprising:

normal cell lines PNT-1 and/or PNT-2 and/or PZ-HPV-7;

alone or together with any or all of one or more cell lines selected from: primary cell lines SHMAC1, SHMAC2, SHMAC5, SHMAC6, P4E6, NIH1519-CPTX, NIH1532-CP2TX, NIH1535-CP1TX or NIH1542-CP3TX;

secondary cell lines LnCap, DU145 and PC3;

alone or together with a non-specific immunostimulant such as BCG, M. vaccae, Tetanus toxoid, Diphtheria toxoid,. interleukin 2, interleukin 12, interleukin 4, interleukin 7.

11. Use of an agent according to any preceding claim in the manufacture of a medicament for the treatment of cancer in humans and other mammals.

12. A method of prophylaxis or treatment of cancer, which includes administering to a patient an agent according to any preceding claim in one or more doses in suitable dosage form.

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Form 23/77: 9/12/99

Agent: Reddie & Grose